bio-protocol Preprint

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Isolation and cryopreservation of gonads

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An abbreviated version of this protocol was published in eLIFE in Jan 2022

A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells

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Detailed protocol

Biobanking of chicken embryonic gonadal tissues

Materials:
Stem-Cellbank: AMS Biotechnology (Europe) Ltd, Cat No. 11897, (20ml)
DMEM
23G (1 ¼" in length) hypodermal needle
Mr. Frosty Freezing Container
One stereomicroscope
100mm Petri dishes
Two sets of forceps and microdissection scissors (sterilised in 70% Ethanol and either air dried or washed in sterile PBS.
Nalgene 1.0 ml cryotubes
Procedure:

1. **Incubation of chicken eggs:** incubate eggs for 9 days (stage 35 HH) in rocking incubator before beginning dissection and isolation of chicken gonadal tissues.

Note: if dissection of gonadal tissue is scheduled for the morning, the eggs will be set up in the morning to allow them developing at expected developmental stage of HH35. A rocking incubator could be an important factor which may affect gonad maturation.

1. **Dissection of gonadal tissues**:
    1. Dissect embryos in a clean laminar flow hood to help maintain sterility during dissection.
    2. Wipe surface of eggs with 70% ethanol to surface sterile the egg shell.
    3. Open the egg's shell from blunt end using forceps and break shell membrane until the embryo body is visible.
    4. Isolate the embryo by placing in a 100mm petri dish, effectively culling the embryo by schedule 1 method. Immediately decapitate the embryo by severing the neck using forceps or scissors.
    5. Under a dissection microscope, position the embryo body by placing the ventral surface (belly) upwards. Cut open embryo using scissors to expose the internal organs. Carefully push the visceral organs using the forceps towards cranial direction to visualize the gonads and the attached mesonephroi lying ventrally to the internal organs. Care must be taken as the gonads are loosely attached to the overlying intestines and may tear free when removing the intestines.
    6. Observe exposed gonads and visually sex the embryo. Males will have two sausage shaped gonads of approximately equal sizes. Females will have a much larger left gonad that will be flattened into a pancake shape.
    7. Gently dissect both gonads off the mesonephros using 23G (1 ¼'' in length) hypodermal needle, use the needle to push the dissected gonads into DMEM which pre-dropped at margin area on the same petri dish to wash off extra blood.
    8. The gonad tissues are then transferred to in a 1.5 ml eppendof tube (screw top) containing 500 µl cold DMEM (one labelled male, one labelled female) and place on ice until a expected pool of gonadal pairs is collected. What size pools?
2. **Cryopreservation of whole gonads**
    1. Place the eppendof tube in a benchtop centrifuge and quick spin for 4 seconds to sink the gonadal tissues to bottom.
    2. Gently remove the DMEM medium on top, try not to disrupt the gonadal tissues, add 100 µl Stem-Cellbanker at RT to the tube for medium exchange, quick spin the tube as 3.1 to re-collect the tissue at tube bottom.
    3. Gently remove supernatant on top and add 200 µl Stem cellbank to the tissue (6 pairs of gonads).
    4. Leave gonadal tissue in Stem cellbank on ice for 15min to equilibrate the tissue, put tubes into Mr. Frosty™ Freezing Container and place in -80°C freezer. (Vitrification of tissues by dropping tube in liquid nitrogen has not been tested).
    5. Transfer the tubes into -150°C freezer or liquid nitrogen next day for long-term storage. Note: long storage at -80C may reduce gonadal PGC survival and migratory competence.

1. McGrew, M. and Hu, J. (2022). Isolation and cryopreservation of gonads. Bio-protocol Preprint. bio-protocol.org/prep1646.
2. Hu, T., Taylor, L., Sherman, A., Keambou Tiambo, C., Kemp, S. J., Whitelaw, B., Hawken, R. J., Djikeng, A. and McGrew, M. J.(2022). A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells. eLIFE. DOI: 10.7554/eLife.74036